

Agricultural Health Study



Demographic, Registry, and Biospecimen Files Users Manual

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1. INTRODUCTION

The Agricultural Health Study (AHS) is a collaborative effort involving the National Cancer Institute (NCI), the National Institute of Environmental Health Sciences (NIEHS), and the U.S. Environmental Protection Agency (EPA). The goals are to investigate the effects of environmental, occupational, dietary, and genetic factors on the health of the agricultural population. This study is providing information that agricultural workers can use in making decisions about their health and the health of their families.

The AHS has four major components:

1. The main prospective cohort study of cancer and noncancer outcomes that includes:
 - a. Linkage with cancer registries and vital statistics registries;
 - b. Ongoing data collection (i.e., telephone interview, food frequency questionnaire, and cheek cell collection (buccal cells));
2. Cross-sectional studies that include (a) questionnaire data, (b) functional measures, (c) biomarkers, and (d) geographic information system (GIS);
3. Nested case-control studies; and
4. Exposure assessment and validation studies.

The AHS cohort includes 89,656 private pesticide applicators, spouses of private applicators, and commercial pesticide applicators recruited within Iowa and North Carolina (Table 1-1). Data for the main cohort study have been collected in phases at approximately five year intervals. Phase I, initial cohort recruitment, began in December 1993 and concluded in 1997. Phase II followup began in 1999 and concluded for private applicators and spouses in 2003. Phase II followup of commercial applicators started in October 2003 and concluded in October 2005. The Phase III followup began in November 2005 and is scheduled to conclude in 2010.

Table 1-1. Composition of Cohort

Type of Respondent	Number Enrolled
Private Applicators	52,394
Spouses	32,346
Commercial Applicators	4,916
Total	89,656

The AHS explores potential causes of cancer and other diseases among farmers and their families and among commercial pesticide applicators. Current medical research suggests that, while agricultural workers are generally healthier than the general United States population, they may have higher rates of some cancers, including leukemia, myeloma, non-Hodgkin's lymphoma, and cancers of the lip, stomach, skin, brain, and prostate. Other conditions, such as asthma, neurologic disease, and adverse reproductive outcomes may also be related to agricultural exposures. The Agricultural Health Study is designed to identify occupational, lifestyle, and genetic factors that may affect the rate of diseases in farming populations.

Iowa and North Carolina were selected for this study based on a nationwide competition. Both states have strong agricultural sectors with diverse production methods, commodities, and products. Information we learn from these two states will be helpful to farmers throughout the United States and other countries using modern agricultural technologies. Field stations in each state administered the questionnaires to respondents in that state and handled all direct contacts with study participants. All survey data are sent to the AHS Coordinating Center where they are assembled into comprehensive files, documented, and distributed to researchers.

Phase I data collection involved administration of questionnaires to pesticide applicators and spouses of private pesticide applicators (i.e., spouses of farmers) to obtain information on pesticide use, other agricultural exposures, work practices that modify exposures, and other activities that may affect either exposure or disease risks (e.g., diet, exercise, alcohol consumption, medical conditions, family history of cancer, other occupations, and smoking history).

Phase II data collection involved administration of a computer-assisted telephone interview (CATI) to cohort members approximately 5 years after their initial enrollment. Following completion of the interview, participants were invited to participate in a collection of buccal (cheek) cells and asked to complete a Diet History Questionnaire. Those who agreed to the buccal cell collection were mailed a kit containing mouthwash, a collection cup, an informed consent form, and a preaddressed, postage-paid mailing envelope. Those who agreed to complete the Diet History Questionnaire were sent a questionnaire and a preaddressed, postage-paid mailing envelope.

Phase III data collection also involved administration of a computer-assisted telephone interview (CATI) to cohort members approximately 5 years after completion of a Phase II interview. The focus of the interviews was to update participants' exposure and health

information. The sampling frame for this effort excluded members of the cohort who fall into one or more of the following categories:

1. Participant completed the Enrollment Questionnaire only, the spouse of the participant is not enrolled, and nothing else was completed during Phase I or Phase II of the study.
2. The participant previously refused all further contact from the Agricultural Health Study.
3. The participant is deceased.

All participants who do not fit into one of the above categories were eligible for the CATI interview in Phase III. This includes all spouses enrolled in the study. Commercial and private applicators are eligible for the study if they: a) completed more than the Enrollment Questionnaire or had their spouse enrolled in the study; b) did not refuse all contact from the Agricultural Health Study; and c) are alive.

Selected participants were asked to provide buccal cell samples using the same collection methods as in Phase II. Those who agreed to the buccal cell collection were mailed a kit containing mouthwash, a collection cup, an informed consent form, and a preaddressed, postage-paid mailing envelope. Only kits with a single buccal cell collection cup were distributed.

Cohort members were tracked passively through state vital statistics offices and the National Death Index (NDI) to determine vital status and, in the case of deceased persons, cause of death. They were also tracked through the Iowa and North Carolina cancer registries in order to record all malignant cancers in the cohort that were reported to the registries. Addresses for individuals not known to be dead and not contacted in the previous year were requested from the Internal Revenue Service. This was done to verify that they were alive and to determine whether they had moved out of Iowa and North Carolina so that person years of followup could be determined.

This manual describes the Demographic, Cancer Registry, Mortality, and Buccal Receipt data files, which are updated annually as part of the main prospective cohort study and are not phase specific. The manual provides the basic information that an analyst needs to use these files. It includes a brief description of the data collection and editing procedures. Detailed codebooks describing each of the variables and the meanings of the recorded responses are

provided as stand-alone documents. The codebooks also contain frequency distributions for each of the variables.

2. DATA COLLECTION PROCEDURES

The source for the data Demographic, Cancer Registry, Mortality, and Buccal Cell Receipt Files varies. The buccal cell collection data come from laboratory receipt files. The cancer and mortality data come from state registries. The demographic data come from questionnaires administered in each phase supplemented by direct phone contact and linkages to various state and national databases. The method for collecting and assembling the data in each of the four files is discussed in separate sections below.

2.1 Buccal Cell Collection

After a respondent completed the CATI questionnaire, he or she was asked to provide a sample of buccal cells which could be used for DNA analyses. The interviewer explained that the procedure involved rinsing the mouth with mouthwash, spitting it into a cup, and mailing it back to the study's buccal processing laboratory. If the respondent agreed to participate, he or she was sent a kit containing an informed consent form, an instruction sheet, a small bottle of Scope mouthwash, a cup for collecting the mouthwash after it had been used to rinse the mouth, and a preaddressed postage-paid mailing envelope. From May 2001 through the end of Phase II, respondents who were known to have cancer were sent kits with two cups instead of one. Those receiving these dual buccal kits were given instructions that requested that they use one on each of two consecutive days. A total of 305 kits containing two cups with buccal samples were received between August 2001 and February 2005.

Respondents placed the completed informed consent form and the buccal collection cup or cups in the mailer and sent them to the processing laboratory in Rockville, Maryland. The laboratory noted the receipt of the consent form and the sample in a receipt file and recorded the amount of material in the specimen. Prior to August 2001, this amount was recorded to the nearest milliliter. Beginning on August 16, 2001, a flag indicating the volume range was used instead. The flags were:

- H (high)
- N (normal)
- L (low)

- V (very low)
- Z (zero).

The volume flags were automatically calculated for all those specimens received prior to August 16, 2001 which had exact measurements recorded, so that a volume flag exists for each cup received. A separate volume flag was stored for each of the cups when dual buccal samples were received.

The lab centrifuged the specimen in each cup separately to extract the buccal cells from the mouthwash solution. The pellet of buccal cells was resuspended in Tris EDTA buffer solution and aliquotted into two vials with approximately 1 to 1.5 ml of the cell suspension. Beginning in April 2001, two vials of the supernatant which remained after the buccal cells from each specimen cup had been centrifuged were also stored. Both buccal cells and supernatant were stored at minus 70 degrees centigrade.

Detailed laboratory procedures are given in Chapter 5. The Buccal Cell Receipt File includes one record for each buccal kit returned by a respondent whether or not it contained a usable sample. Freezer location data for each vial are available through the National Cancer Institute's Biological Specimen Inventory-II system (CBSI-II).

2.2 Cancer Registry Data

Cancer Registry data are obtained annually from the Iowa and North Carolina cancer registries by the field stations in each state. Each field station submits a list of participants to its respective cancer registry late each year and then reviews the matches returned to ensure that the data entered into the AHS files represent members of the cohort. Once the files have been adjudicated, they are forwarded to the Coordinating Center and combined into a single data file.

The Surveillance, Epidemiology, and End Result (SEER) site recode variable (SITEREC) is then added to the file. The values of the SITEREC variable are based on the primary site and histology data fields. These values represent the major cancer sites that are commonly used in the reporting of cancer incidence data. When the cancer is coded using ICD-O-2, the values of SITEREC use the "SEER Site Recode ICD-O-2 (1/27/2003) Definition," which can be found at http://seer.cancer.gov/siterecode/icdo2_d01272003/. When the cancer is coded using ICD-O-3, the values of SITEREC use the "SEER Site Recode ICD-O-3 (1/27/2003) Definition," which can be found at http://seer.cancer.gov/siterecode/icdo3_d01272003/. The

ICD-O-2 and ICD-O-3 definitions of site recode include the same set of site groups and are comparable to one another.

2.3 Mortality Data

Mortality data are obtained annually from the Iowa and North Carolina vital statistics offices by the field stations in each state. Each field station submits a list of IDs to its respective state office late each year and then reviews the matches returned to ensure that the data entered into the AHS files represent members of the cohort. Each field station also submits a list of all cohort members not known to be deceased to the Coordinating Center. These lists are combined and submitted to the National Center for Health Statistics to compare to the latest available annual National Death Index (NDI). The NDI results for each field station are returned to the field station by the Coordinating Center. They are then adjudicated and added to the state's mortality file. The NDI searches ensure that deaths of cohort members who die out of state but within the United States are reflected in the AHS Mortality File.

Once the state mortality files have been completed, they are forwarded to the Coordinating Center and combined into a single data file.

2.4 Demographic Data

The Demographic data come from a number of sources. They represent the best available demographic and administrative information the AHS has about a cohort member. Some variables such as state of residence are updated in field station tracking files whenever new information comes to the field station. Other data (e.g., race and ethnicity) were collected at enrollment through either the Enrollment Questionnaire (for applicators) or the Spouse Questionnaire (for spouses of private applicators). Variables representing which questionnaires have been completed are derived from the latest version of the corresponding AHS data files. High level cancer and mortality data reflect data in the Cancer Registry and Mortality Files. The field stations send extracts from their tracking files to the Coordinating Center annually. These files are combined into a single file and the data from other project files is merged in to complete the Demographic File.

When race and ethnicity values were not available from the Phase I questionnaires collected at enrollment, data in the Cancer Registry File and the Mortality File were used to

augment the race and ethnicity variables in the Demographic File. The following hierarchy was used when more than one value was available:

1. Values from the Phase I questionnaires;
2. Values from the Mortality file; then
3. Values from the Cancer Registry File.

The derived variables RACE_SOURCE and HISPAN_SOURCE document the source of the race and ethnicity data. For each variable, the possible values for source are:

- 1 -- Phase 1 questionnaires files;
- 2 -- Mortality File; and
- 3 -- Cancer Registry File

3. DATA FILE DESCRIPTIONS

The study IDs of the respondents can be used to link all of the AHS data files including the phase-specific interview files and the annually updated files. The following files represent the Demographic, Registry and Biospecimen data:

- Buccal Cell Receipt File
- Cancer Registry Data File
- Mortality Data File
- Demographic Data File

A separate codebook exists for each of these files that provides a detailed description of its contents. Each of the files is discussed briefly in a separate section below.

3.1 Buccal Cell Receipt File

The Buccal Cell Receipt File contains the receipt records of buccal cell collection kits sent to the processing laboratory. Kits were entered into the receipt file by the laboratory whether or not they contained usable samples. The file provides the date that a collection kit was received, information on whether a completed informed consent form was received for the specimen, a measure of the gross volume of the submitted sample, an indicator for whether the sample was damaged, a flag indicating whether one or two collection cups were sent by the subject, and comments about the sample or the consent form as needed.

There are 36,866 records in the file representing buccal collection kits that were received between January 22, 1999 and May 2009. This file represents all the kits returned as a result of the Phase II interviews and the kits returned during the Phase III data collection through May 2009. The distribution of records in the file is shown in Table 3-1.

Table 3-1. Distribution of Applicator Types in Buccal Cell Receipt File.

Type of Applicator	Number of Buccal Kits Returned
Private applicators	20,448
Spouses of private applicators	14,503
Commercial applicators	1,915
Total	36,866

In some cases when a participant was called by a field station and reminded to send back a buccal collection kit, the participant requested a replacement kit. Some of these people eventually sent in both the original kit and the replacement. Some people who provided buccal specimens during Phase II of the study also provided buccal specimens during Phase III. For these reasons the total number of unique IDs in the Buccal Cell Receipt File (N = 35,243) is lower than the total number of kits represented in the file.

3.2 Cancer Registry File

The Cancer Registry File contains data on cohort members with cancer. This information was collected by the Iowa and North Carolina cancer registries and identified cancers diagnosed and their corresponding diagnosis dates. The Iowa and North Carolina field stations searched their state cancer registries to identify records that matched members of the AHS cohort and forward the matches to the Coordinating Center.

The State Health Registry of Iowa is a member of the Surveillance, Epidemiology, and End Result (SEER) program sponsored by the National Cancer Institute. SEER collects data from nine U.S. geographic areas to provide a representative sample of cancer in the United States. Since cancer diagnosis dates are reported to the SEER as month and year only, the diagnosis dates for Iowa have been set to the 15th of the month in the AHS Cancer Registry File.

The North Carolina Central Cancer Registry is a state population-based registry that is not part of the SEER system. This registry receives and reports complete diagnosis dates.

An individual may be represented by more than one record in the Cancer Registry File because of having multiple cancers. Each record contains basic demographic information about the participant and cancer information including the diagnosis date; a flag indicating whether or not the cancer occurred after enrollment in the study; and ICD codes for cancer site, laterality, morphology, and up to 3 tumor markers. The records also contain information on each cancer's behavior, stage, and grade.

There are 10,323 records in the file. The data represent nearly complete cancer diagnoses through 2006 and some diagnoses in 2007 (N = 331) and 2008 (N = 49).

3.3 Mortality Data File

The Mortality Data File contains dates and causes of deaths of AHS cohort members. The Iowa data were obtained by the Iowa field station matching records to those of the Iowa Department of Public Health Bureau of Vital Records data files. The North Carolina data were obtained by the North Carolina field station matching records to the Detailed Death Master file compiled by the North Carolina Center for Health Statistics Vital Records Unit. Both the Iowa and North Carolina data contain death records through calendar year 2007. The file also contains some (N = 192) Iowa death records from 2008.

The Iowa and North Carolina state data were supplemented by reviewing the results of a National Death Index (NDI) search. The NDI search included data from all U.S. states through December 2006. There are 6,611 records in the Mortality File.

3.4 Demographic Data File

The Demographic File contains basic demographic information about members of the cohort including birth date, gender, race, ethnicity, highest level of schooling (at enrollment), marital status at enrollment, vital status, and in-state status. There is one record in the file for each member of the AHS cohort. Each record contains a flag indicating whether a person has been diagnosed with cancer and an indicator of whether this is an incident cancer or not. For deceased persons, the file contains the date of death and the date of last contact by the field station.

The file also contains a number of administrative variables including enrollment date, age at enrollment, flags indicating whether or not the individual completed each of the possible Phase I questionnaires, the Phase II interview status code assigned by the field station, Phase II CATI start date, age on the day that Phase II CATI was started, and a flag indicating whether or not a Phase II respondent has data in the Supplemental Pesticide file, which indicates whether or not information was collected on the person's use of specific pesticides.

Some variables such as birth date, gender, race, and ethnicity appear in other data files as well as in the Demographic File. When these differ, the data in the Demographic File have been updated with the most current information. The other values are what were provided at the time of the interview. When there is a difference, the values in the Demographic File should be used for analytic purposes.

There are 89,656 records in the Demographic File.

4. CODEBOOKS

Codebooks for each data file are available as stand-alone documents. For each variable in the dataset, the codebook lists the variable name, indicates whether it is character or numeric, lists the question for items drawn directly from a questionnaire or provides a description for derived variables, and gives the range of valid values for each variable. For categorical variables, the codebook also specifies the meaning of each value. Frequency distributions are provided for each variable.

The codebooks are:

- Buccal Cell Receipt File
- Cancer Registry File
- Mortality File
- Demographic Data File

5. LAB PROCEDURES

The project used one laboratory to process the buccal specimens from the inception of buccal processing collection through August 15, 2001, and changed to a new laboratory effective August 16, 2001. The protocol used by the first laboratory is included in Section 5.1. That of the second laboratory is included in Section 5.2.

5.1 First Laboratory Protocol

Note: The formatting and numbering of the procedural steps below is that used by the laboratory.

Specimen Processing of Buccal Cells for the Agricultural Health Study

Purpose

This document outlines the procedures for the processing of the buccal cell samples according to the guidelines of the Agricultural Health Study

Definitions

AHS: Agricultural Health Study

MI: milliliter

RLIMS: Relational Laboratory Information Management System

BSC: Class II Biological Safety Cabinet

EDTA- Tris Buffer

Scope

This procedure will be followed by all laboratory personnel handling specimens for the Agricultural Health Study.

Specimen(s)

The specimens that are collected for this study are from farmers who live in two different geographical locations (Iowa and North Carolina). These farmers are given a kit with a small plastic container and a small bottle of mouthwash. The farmers brush their teeth and then rinse their mouth with the mouthwash and deposit it into the container that is provided. In this specimen, buccal cells are collected

Supplies, Equipment, Reagents, Media - required for testing

- 1) 10 ml pipettes
- 2) 5 ml pipettes
- 3) ml pipettes
- 4) 15 ml centrifuge tubes
- 5) EDTA Tris- Buffer
- 6) 2 ml Cryovials
- 7) Freezer boxes
- 8) Freezer grids
- 9) Labels
- 10) Sample location sheets
- 11) Cryovial racks
- 12) Juoan Centrifuge

Specimen Procedures

Accessing Buccal Samples

- 1) The samples are sent to the laboratory from two sites, Iowa and North Carolina, via US mail.
- 2) Upon arrival, samples are taken into walk-in freezer for storage prior to processing.
- 3) When the sample is taken into the laboratory the envelope is opened to check for the participant's consent forms.
- 4) A barcode is placed on the envelope in which the sample came such that the sample can be scanned into RLIMS.
- 5) While still in the envelope, the sample is then put in a bin so that they can be processed in batch.
- 6) When ready to process, the samples are then taken to the computer and logged into the computer by scanning the bar codes.
- 7) Scan sample into RLIMS
- 8) Save the information that was scanned in.
- 9) Once all the samples have been scanned into RLIMS they are ready to be processed.
- 10) The samples are kept in the walk-in freezer until they are ready to be processed.
- 11) The barcode that is on the envelope can be taken to the computer and labels can be made by using the bar code scanner. 8 labels should be created.
- 12) Once labels are made for all of the samples, you take the samples to the walk-in freezer.

Processing the Buccal samples

- 1) The samples are taken out of the walk-in freezer into the laboratory.
- 2) Once in the laboratory, take the samples to the BSC and take the labels out of the bin.
- 3) Label the 15 ml centrifuge tubes and the sample location form.
- 4) Label cryovials.
- 5) Take the sample out of the envelope.
- 6) Match the number on the envelope with the number on the plastic container that has the buccal sample. Ensure the numbers on the envelope and the plastic container match the number that was assigned to the sample.
- 7) Place two labels on the vials for the supernatant and a red insert cap will be placed in the vial. Place a "supernatant" label on these vials.

- 8) Place two labels on the cryovials used to store buccal cells along with another label which says buccal cells.
- 9) Open the plastic container that contains the buccal sample and aspirate the sample three times using a 10 ml pipet.
- 10) Measure out the amount of fluid that is in the container. Record this measurement on the data sheet.
- 11) Take the sample to the Juoan centrifuge in a test tube rack. Program the centrifuge for program for 15 minutes at 500 rpm. Do not let sample sit for more than 10 mins. In the centrifuge after spin.
- 12) Once the sample has finished its cycle in the centrifuge take them all out and transfer to a test tube rack.
- 13) Put the sample into the BSC and take off the supernatant in each tube and put 1.8 mls of supernatant into each of the 2 cryovials that are labeled for supernatant.
- 14) Once the supernatant is discarded, take a 5 ml pipet and get 3 ml of EDTA-Tris Buffer.
- 15) Mix the cells up by aspirating about 4 times.
- 16) Open up the cryovial and dispense 1 ml per cryovial into 2 vials.
- 17) Fill the data sheet out for the cell location.
- 18) Turn data sheet into the data information group.

5.2 Second Laboratory Protocol

Note: The formatting and numbering of the procedural steps below is that used by the laboratory.

Processing of AGRICULTURAL HEALTH STUDY Buccal Cell Samples

- 1.0 Purpose: To describe the receipt, processing and storage of buccal cell samples for the AGRICULTURAL HEALTH STUDY.
- 2.0 Policy: The receipt, processing, and storage will comply with Good Laboratories Practices (GLPs)
- 3.0 Equipment:
 - 3.1 IEC Centra-8 or Forma Scientific Centrifuge
 - 3.2 Biological Safety Cabinet
 - 3.3 Refrigerator
 - 3.4 -80°C Freezer
- 4.0 Materials:

- 4.1 50 ml conical centrifuge tube
 - 4.2 5.0 ml disposable sterile serological pipettes
 - 4.3 Tris EDTA buffer solution pH 8.0 (Quality Biological Inc. #351-0110131)
 - 4.4 2.0 ml sterile cryovials
- 5.0 Procedure:
- 5.1 Specimen Receipt Procedure
 - 5.1.1 Open and remove two specimen collection cups from participant's envelope. An Informed Consent form should be included with the specimens. Review and ensure participant has signed and dated form.
 - 5.1.2 Attach a specimen identification label (AG+5) on the Informed Consent Form.
 - 5.1.2.1 The Informed Consent Forms are copied, sorted by site, and sent to sites each week. Receipt of the Informed Consent Forms will be confirmed by the sites and copies will be sent to BioReliance for destruction.
 - 5.1.2.2 An Informed Consent Form may be received separately from the collection cups. In the receiving database, change the Informed Consent Form field to a "Y" to reflect receipt of this form. Sites will notify McKesson by E-mail if they receive an Informed Consent Form. Change the Informed Consent Form field in the receiving database to a "Y" to reflect receipt of this form. E-mail the site the AG number associated with the Informed Consent Form.
 - 5.1.3 Buccal cell samples for the AGRICULTURAL HEALTH STUDY should be processed on the day of receipt. If this is not possible, the samples are to be stored in the refrigerator until they are processed.
 - 5.1.4 Receive samples by accessing the Biological Inventory System (BioSIS) and selecting the Agricultural Health Study Receiving Module.
 - 5.1.5 Enter the following information into the Agricultural Health Study database :
 - 5.1.5.1 Enter Study Number (AHS Buccal)
 - 5.1.5.2 Enter date envelope received
 - 5.1.5.3 Did participant refuse to provide a specimen (Y or N)?
 - 5.1.5.4 Were dual cups received (Y or N)?
 - 5.1.5.5 Enter the estimated volumes in the collection cups.

Z- None: Below 1.0 ml
V- Very Low: 1.0 ml to 5.9 ml
L- Low: 6.0 ml to 10.3 ml
N- Normal: 10.4 ml to 17.5 ml
H- High: Above 17.5 ml

- 5.1.5.6 Was the sample damaged or spilled (Y/N)?
- 5.1.5.7 Was the Informed Consent Form received (Y/N)?
- 5.1.5.8 Did the participant sign the Informed Consent Form (Y/N)?
- 5.1.5.9 Enter the date the Inform Consent Form was signed.
- 5.1.5.10 Enter comments concerning the Informed Consent Form or specimen.
- 5.1.5.11 Scan the patient barcode on the Informed Consent Form.
- 5.1.5.12 Scan the barcode(s) on the collection cup(s).
- 5.1.5.13 Scan the barcode on the Specimen Identification label.

5.1 Specimen Labeling Procedure

- 5.2.1 Generate labels using the AGRICULTURAL HEALTH STUDY Label Generation Program. Ten labels are generated per participant; five per collection cup received. Label information is listed below:

Label Affix To	Label Information
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No #	Centrifuge Tube AG +5 digits and collection cup ID
# 01	Cryovial- Cells AG +5 digits-1 and collection cup ID
# 02	Cryovial- Cells AG +5 digits-2 and collection cup ID # 03
	Cryovial-Supernatant AG +5 digits-3 and collection cup ID
# 04	Cryovial-Supernatant AG +5 digits-4 and collection cup ID
No #	Centrifuge Tube AG +5 digits and collection cup ID
# 05	Cryovial-Supernatant AG +5 digits-5 and collection cup ID
# 06	Cryovial-Supernatant AG +5 digits-6 and collection cup ID
# 07	Cryovial-Cell AG +5 digits-7 and collection cup ID
# 08	Cryovial-Cell AG +5 digits-8 and collection cup ID

- 5.2.2 Label the centrifuge tubes and vials.
- 5.2.3 Move the collection cups and centrifuge tubes into the Biological Safety Cabinet and prepare for processing.

5.3 Specimen Processing Procedure

- 5.3.1 In the Biological Safety Cabinet, invert the collection cups 4 to 6 times before removing the top. Check labels on the centrifuge tubes. The ID number on each centrifuge tube must match the ID number on the collection cup. Transfer each specimen into a separate centrifuge tube. If a participant sends in two cups, each cup must be placed in a separate centrifuge tube. Continue until all specimens have been transferred.

- 5.3.2 Place the centrifuge tubes into the IEC Centra-8 or Forma Scientific centrifuge. Centrifuge at 1500 x g for 15 minutes (3100 RPM), with the break off.
- 5.3.3 Move the centrifuge tubes and the corresponding cryovials into the Biological Safety Cabinet. Before starting, make sure the AG number on the centrifuge tube corresponds to the AG number on the cryovials.
- 5.3.4 Using a sterile 5 ml pipette, remove 3.6 ml of the supernatant. Place 1.8 ml of supernatant into each of the two cryovials #3 and #4. If applicable, the supernatant from the second collection cup will be placed into cryovials #7 and #8. Change pipettes if a second collection cup is used.
- 5.3.5 Discard the remaining supernatant from the centrifuge tube.
- 5.3.6 Using a 5 ml sterile pipette, add 3 ml of Tris EDTA pH 8.0 to the centrifuge tube. Disperse the buccal cell pellet by gently tapping the 50 ml tube and then resuspend using the pipette.
- 5.3.7 Remove the entire sample from the tube and aliquot equal amounts (approximately 1.5-1.8 ml) into two 2.0 ml sterile cryovials label #1 and #2. If applicable, the cells from the second collection cup will be placed into cryovials #5 and #6.
- 5.4 Specimen Inventory Procedure
 - 5.4.1 Access the Biological Specimen Inventory System (BioSIS) and select the AGRICULTURAL HEALTH STUDY Inventory Module.
 - 5.4.2 Enter the date of processing into the inventory system.
 - 5.4.3 Scan the barcodes on the cryovials into the inventory system. As the vials are scanned, the computer will automatically assign the location codes.
 - 5.4.4 Place the cryovials into the -80°C freezer.
- 5.5 Specimen Data File Transfer Process
 - 5.5.1 At the end of each day, generate the receiving file. This file is electronically sent to Westat via FTP. At the end of the month, the entire receiving and inventory databases are sent to Westat via FTP. Send Westat an E-mail message confirming that the files have been transferred.